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CONTRIBUTIONS
No. 31

The Melanconis Disease of the Butternut (Juglans cinerea L.)

BY

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BROOKLYN, N. Y.
1923

Reprinted, without change of paging, from PHYTOPATHOLOGY
13: 411-435, October, 1923

THE MELANCONIS DISEASE OF THE BUTTERNUT (JUGLANS CINEREA L. [1]

ARTHUR HARMOUNT GRAVES

WITH PLATES XIX AND XX AND 5 FIGURES IN THE TEXT

INTRODUCTION

For about twenty years the writer has been particularly interested in the general pathological condition of the butternut, (*Juglans cinerea* L.). During this period several trees in Connecticut of various ages, on which observations were made from year to year, have finally died, and others have been gradually growing weaker. (Pl. XIX, figs. 1, 2). Previous to a note by the writer, in 1919, (8) there was no mention in the literature of any serious trouble; although the leaf spot caused by *Gnomonia leptostyla* (Fr.) Ces. and De Not., (the perfect stage of *Marssonia Juglandis* (Lib.) Sacc.) has been reported to sometimes cause considerable damage (14, 19), and also the basidiomycetous wood-rotting fungi doubtless do not neglect this tree (14).

A thorough investigation of the cause of the trouble was begun in the summer of 1918, during a survey of diseases of trees in Greater New York. In this region, and in the same year in Pennsylvania and Maryland, the butternuts were in such a weakened condition as to compel attention. At this time the acervuli of an imperfect fungus, *Melanconium oblongum*, were found to be of quite general occurrence on the dead branches, and this organism was therefore tentatively assumed to be the cause of the trouble, particularly when greenhouse experiments with healthy twigs inoculated with the fungus in question gave positive results.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THE DISEASE

In many of the excellent "tree books" now current, one finds statements like the following:—

"Trees of the same age are apt to vary in vigor and size, dead branches are likely to appear early, and sound trees 8 or 10 inches in diameter are seldom seen;" (5) or,

[1] Brooklyn Botanic Garden Contributions, No. 31.

"While the butternut is often a very attractive tree as seen growing spontaneously, it is not to be depended upon for ornamental planting, as it is very apt to develop dead branches which interfere with its symmetry. Good-sized trees, even when growing under favorable conditions, generally show this disfigurement, and larger trees are very likely to die completely before they attain any great size." (7)[1]

Dr. G. P. Clinton tells us that he has noticed the weakened condition of the butternut in Connecticut for a number of years, and calls attention to it in a recent bulletin (4), citing the work of the writer. Dr. L. O. Overholts, who recently collected the causal fungus in the mountains near Pennsylvania State College, writes that it "certainly has the appearance of being parasitic in this instance." Prof. J. Franklin Collins has noticed sickly trees in Rhode Island, and in 1922 sent us specimens of both perfect and imperfect stages of the fungus. Dr. C. H. Kauffman writes that he has made collections in New York and Maryland; and likewise Dr. A. S. Rhoads, in New York.

This testimony, taken in connection with the observations of the writer in Connecticut, New York, Pennsylvania, and Maryland, and with his study of exsiccati (p. 415) of the fungus in question, i. e. *Melanconium oblongum* Berk., and its perfect stage *Melanconis Juglandis* (E. & E.) comb. nov., on butternut, certainly justifies the conclusion that the disease is widespread, and very probably extends over the entire natural range of the butternut.

The disease is important *per se*, because it eventually brings about the death of the tree, although its progress is slow. During its course, more and more twigs and larger branches are killed, and this means, naturally, a progressive loss in the total photosynthetic activity. Typical, rather young trees, about 30 years of age, have been noted in the forest with approximately one third of all their large branches dead. Such a loss cannot fail to have a reactionary, weakening effect on the whole tree. But many trees have also been observed which are almost entirely dead while still young (10–20 years). Older trees with only a few living branches remaining are common. (Pl. XIX, fig. 2). In general, taking into account the shortening of the life of the tree as well as its slower growth due to this disease, we may perhaps estimate the loss of normal healthy growth of butternut timber to be somewhere between 30 and 50 per cent.

[1] The following extract from a letter from Dr. Robert T. Morris, the well-known nut culturist, is of interest: "Over the greater part of Connecticut when I was a boy we had magnificent butternut trees, and on my country place at Stamford the last one of the majestic ones is now dead. Most of the younger generation died before they were thirty years of age, many of them at fifteen or twenty years."

We do not believe however, that there is danger of disappearance of the butternut, due to the activities of the fungus associated with this trouble, as has been claimed. (13) The disease ordinarily progresses so slowly that there is ample opportunity for reproduction of the tree and thus for perpetuation of the species.

But while the present paper is confined mainly to the disease in its relations to the butternut, the trouble is of vastly greater significance in its potentiality with regard to other species of Juglans. The fungus has been collected by the writer on *Juglans nigra*, one of our most valuable species of hard woods, and preliminary work (p. 431) has shown that other species of Juglans are susceptible.

From a larger, practical standpoint, the economic importance of the disease in the butternut is bound up with the economic value of the butternut tree itself.[1]

Besides its timber, which is much esteemed for certain purposes, the tree has several well-known minor uses (13, 17), so that a disease which seriously threatens it should be regarded as of considerable economic importance.[2]

SYMPTOMS OF THE DISEASE

It should be emphasized first of all that the progress of the disease, in typical cases, is very slow. No sudden wilting of the leaves occurs —nor does any well marked lesion or canker, as in the chestnut blight, develop on infected branches.

From observations extending over a period of three years made on 62 trees in various localities in Connecticut and New York, the course of the disease is essentially as follows: small branches, either from the sides of the trunk, or from a larger branch, die slowly in basipetal succession, the disease to all outward appearances proceeding from the distal end of the branch toward the trunk. If this happens during the

[1] A botanist of some standing, in the course of a conversation with the writer about this disease, remarked that our country could well get along without the butternut. With the same line of reasoning one may argue that many of our native timber trees of secondary commercial importance may be dispensed with, and thus eliminate them, one by one. But we are losing all of our chestnut, a tree of the first magnitude; the white pine, the most valuable coniferous species in the Eastern United States, is seriously threatened; and because of the increasing scarcity of better woods many species formerly considered of minor importance, are coming into service, and have a constantly rising value. Certainly this policy of *laissez-faire* as voiced by our colleague, is greatly to be deplored and is diametrically opposed to the principle of conservation.

[2] Butternut is included in the list of 100 important eastern forest trees in "Important Forest Trees of the Eastern United States." U. S. Dept. of Agr. Dept. Cir. 223. 1922.

growing season, the leaves of the branches in question turn yellow gradually and drop off—in such slow succession, however, as to pass unnoticed by the casual observer—or the gradual death of the branch may occur in the leafless season. The bark of the dying branches changes from its natural dark greenish brown tinge to a reddish brown and finally a dull gray. Soon it becomes studded with small, dark, smooth papillae, which at length burst open exposing the black pustules or acervuli of the fungus, (Pl. XX, figs. 3, a, b, c). A year or so later all that remain of the pustules are small rounded or irregular holes in the loose outer bark (Pl. XX, fig. 7).

The disease is characterized by a succession of new, rather short branches which die in their turn, developing farther and farther down the main branches or leaders. Occasionally a crown of short lived suckers which soon become infected, appears on the trunk itself. In the last stages of the disease the tree is marked by the bare, gray, weathered wood of dead branches in the upper part, giving a characteristic stag-headed aspect (Pl. XIX, fig. 2). At this period, too, basal shoots often arise which soon become infected.

THE FUNGUS

Synonymy

In its imperfect form, the fungus associated with the disease has been known since Berkeley first described it from specimens from the United States in 1874 (2) as *Melanconium oblongum*. This is the stage which is usually found on diseased butternut and other species of Juglans. In 1893 Dr. Shear collected another form in close proximity to the conidial form on dead branches of butternut, and according to a note by him in the herbarium of the New York Botanical Garden, he believed it might be the perfect stage of *Melanconium oblongum*. In the same year Ellis and Everhart named this form *Diaporthe Juglandis* from Shear's material, citing his collection. Since the writer, by culture work (p. 420), has established beyond question that *Diaporthe Juglandis* E. & E. is the ascogenous stage of *Melanconium oblongum*, it seems advisable to assign the fungus to the genus Melanconis of the family Melanconidaceae, for this group is characterized by superficial conidia (except in Valsaria) of the type of Melanconium. *Melanconis carthusiana* Tul. was long ago (12) connected with *Melanconium juglandinum* Kze., which is of common occurrence on *Juglans regia* in Europe. This conidial form is quite similar to *Melanconium oblongum*, but a careful examination and comparison of exsiccati of the two forms has convinced us that they are

distinct. The following new combination is therefore proposed for this American species of Melanconis, and a description is here added to supplement the characterization of *Diaporthe Juglandis* by Ellis & Everhart. (6)

MELANCONIS JUGLANDIS (E. and E.) comb. nov.

Perithecia seated in scanty stroma ⅓ to ½ mm. below surface of bark, in groups of 1–9, usually not more than 5, sometimes 1, globose, or slightly flattened tangentially, black, 500 μ in diameter, (range 441 × 617 μ) their necks black, about 90 μ in diameter, and ordinarily only slightly extruded; asci cylindrical or slightly club shaped, 108 × 11.7 μ (range 90–110 × 10.5–12 μ), with a short stipe about 6 μ long (range 3–9 μ); ascospores hyaline, colorless to light grayish olive—(15, Pl. XLVI), elliptical, with blunt ends or sometimes pointed, 2-celled and slightly constricted at the septum, averaging 20 × 8.5 μ.

Pycnidia developing in culture, black, spherical or irregular, partly sunk in the medium, 150–400 μ in diameter, with a rounded apical pore, and surrounded by a dense felt of mycelium. Pycnospores oblong, hyaline, one-celled, 1 × 3 μ, swelling markedly on germination.

The conidial form is *Melanconium oblongum* Berk. with usually inequilateral spores, averaging 19 × 9 μ, olive gray (15, Pl. LI) under the microscope, but appearing jet black when massed in the acervulus.

Habitat. North America.[1] A slow parasite in wood and bark of *Juglans cinerea* L. and *J. Sieboldiana* L.; also occurring on *J. nigra* L. and *J. regia* L. and perhaps pathogenic there.

HOSTS AND LOCALITIES OF OCCURRENCE

In the following list, which makes no pretensions as to completeness, all specimens except those starred have been examined by the writer. On *Juglans cinerea.*

CANADA: London, May 13, 1892; no definite locality, February 11, 1893, "*Macoun.*"

MAINE: North Windham, July 26, 1923, *A. H. G.*

RHODE ISLAND: Lincoln, September 30, 1922, (perfect stage) *J. Franklin Collins.*

CONNECTICUT: West Hartford, September 24, 1919, *A. H. G.*; Meriden, September 16, 1922; Newington, September 16, 1922; Berlin, September 16, 1922; Hamden, January, 1919; July 2, 1920; October 21, 1920 (perfect stage); September, 1921; January 3, 1922; September 16, 1922 (perfect

[1] In the list of Danish fungi in Dr. Rostrup's herbarium (11) one specimen of *Melanconium oblongum* is included with the statement that it occurred on *Juglans mandshurica* in the garden of an Agricultural High School and is new for Europe.

stage), all *A. H. G.*; North Branford, December 19, 1920, *A. H. G.*; Sharon, July 12, 1920, *A. H. G.*; Southport, September 27, 1922, *A. H. G.*; Norwalk, September 27, 1922, *A. H. G.*

NEW YORK: Farmington, July, 1889, *E. Brown*; Canton, 1857, (no collector given); also a second specimen from this same locality without date or collector; Buffalo, no date, *G. W. Clinton*;* Alcove, August 1893, "with Diaporthe juglandis E. & E.," *C. L. Shear*; Potsdam, no date, (N. A. F. 121); Van Cortlandt Park, New York City, August 16, 1918, *A. H. G.*; Cold Spring Harbor, August 14, 1918, *A. H. G.*; Inwood, New York City, January 3, 1919, *A. H. G*; Baldwin, L. I., December 6, 1921, *A. H. G.*

NEW JERSEY: Newfield, June 1892. (*Fungi Col. 153*).

PENNSYLVANIA: Kennett, September, 21, 1918, *A. H. G*; Shingletown Gap, Center County, September 26, 1922, *L. O. Overholts.*

MARYLAND: Glen Echo, January 9, 1919, *C. H. Kauffman.**

WEST VIRGINIA: Belleville, December 27, 1921, *Lee Bonar.**

WISCONSIN: St. Croix Falls, November 1, 1897, *C. F. Baker.*

IOWA: No definite locality, May 1882, *E. W. Holway.*

On *Juglans nigra.*

NEW YORK: Valley Stream, L. I., April 1921, *A. H. G.*; Baldwin, L. I., December 16, 1921, *A. H. G.*

On *Juglans Sieboldiana.*

CONNECTICUT: Stamford, July 20, 1919, *A. H. G.*; New Haven, July 31, 1920, *A. H. G.*

NEW YORK: Ithaca, campus of Cornell University, March 28, 1904, *C. H. Kauffman*;* Baldwin, L. I., December 16, 1921, *A. H. G*; Brooklyn, (from inoculated seedlings), June 12, 1922, *A. H. G.*

On *Juglans regia.*

CONNECTICUT: Stamford, July 20, 1919. *A. H. G.*

NEW JERSEY: Newfield, 1892, *Ellis* (N. A. F.).

MORPHOLOGY OF THE FUNGUS

Mycelium

In a culture two days old on nutrient medium, the hyphae are hyaline, colorless and copiously branched, the main trunks being from 6–8 μ in diameter (fig. 1, a, b). The cross walls are distinct, the hyphae being often slightly constricted at these points. These numerous branches, constrictions, and also frequent, short, knubbly branches give the young mycelium a characteristic ragged aspect. Various colors and color changes develop under the influence of different culture media, but in old

cultures the prevailing shades are olives and grays. Under the microscope, old mycelium grown in oat agar has a yellow-green shade. In old cultures the main hyphal trunks are of about the same diameter as in the young cultures, but there may be a host of many fine hairlike branches about 2μ in diameter with cross walls indistinct. Between these two extremes, all gradations exist. In connection with the development of conidia and pycnidia in cultures, a smoke gray, fluffy, aerial mycelium

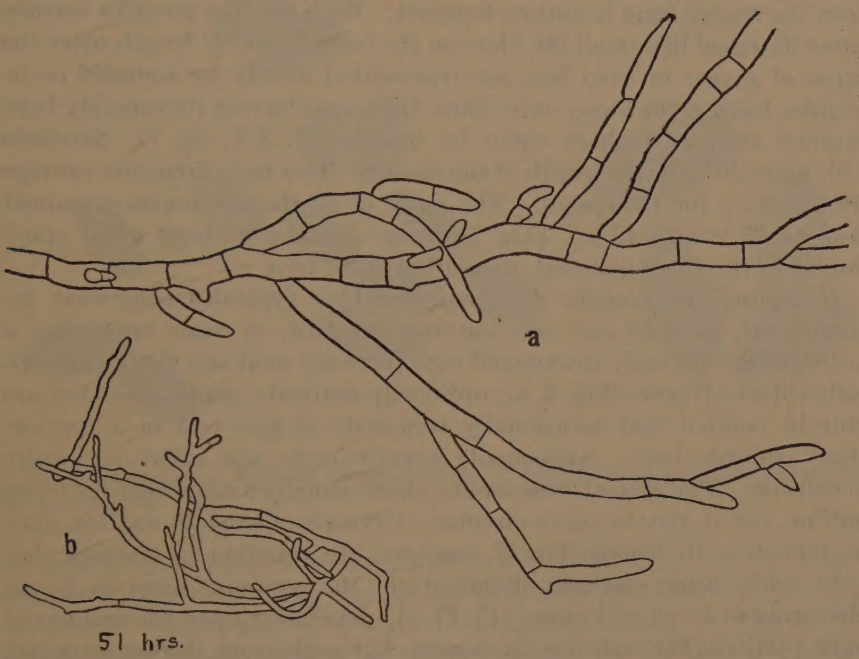

Fig. 1. Mycelium from a young culture: a, 2½ days old, × 410; b, 51 hours old, × 110.

appears, often in cushionlike tufts. This is composed of very fine hyphae essentially similar to the fine branches in the medium. In the bark of the host, the mycelium is even more slender—from 1 to 1.5 μ in diameter.

Conidial Stage

It is this form which is almost invariably seen on dead butternut branches, at first developing small, rounded, pimply, blackened elevations, scattered irregularly about (Pl. XX, fig. 3, a, b) and later bursting through the epidermis and young cork layers with rounded, irregular openings (Pl. XX, fig. 3, c) revealing the pustules of the apparently inky-black conidia. Although pustules appear usually only on the twigs

and small branches, occasionally they are found on the thick bark of the trunk (Pl. XX, fig. 4); and as shown by the figures (Pl. XX, figs. 5, 8, 9) there is much variation in their size. Sometimes they are elongated or run together (Pl. XX, fig. 9). If the weather is damp or rainy, they resemble tiny drops of thick ink, or if the twigs are placed in moist chambers, with the humidity of the correct percentage, the conidia are extruded in tendril form (Pl. XX, fig. 5). We have rarely seen the tendril form in nature, however. With age, the pustules become more flattened like small ink blots on the branch; and at length, after the lapse of a year or even less, are represented merely by rounded or irregular holes in the loose, outer bark, the spores having presumably been washed away or perhaps eaten by insects (Pl. XX, fig. 7). Saccardo (16) gives 20 μ for the length of the conidia. Our measurements average 19.1 × 9.3 μ for 150 spores. The range of all the specimens examined was 14–25 × 4.25–13 μ. This, however, excludes a single giant spore found in the Iowa material, measuring 30 × 13 μ.

In shape, the conidia vary considerably; typically somewhat inequilateral, or with one side curving outward, in some specimens a kidney shape prevails, intermixed with perfectly oval and also symmetrically elliptical forms (Fig. 2, a); ordinarily muticate, sometimes they are bluntly pointed and occasionally terminate at one end in a narrow, short truncate base. Abnormally curved forms also occur, especially in cultures. The optical cross section shows usually a somewhat flattened outline, but it may be quite circular. Crystals of calcium oxalate, such as Johnston (9) records for *M. sacchari*, are common in the pustules.

In color, using daylight illumination, the contents seem to be an olive gray to a deep olive gray (15, Pl. 51) depending upon the amount of light admitted through the diaphragm, but with much darker brownish walls; in fact, it is the wall which gives the dark color to the spore as can be determined by crushing. The contents are then shown to be in reality much paler, though still a shade of gray. Spore specimens which have been kept in glycerine mounts for three years have retained their natural color well.

As to contents, the conidia are densely packed with light-refracting granules, and sometimes contain one droplet or several small ones (Fig. 2, b).

As indicated above, there is some variation in size in the material from different localities, that from Iowa being the largest, averaging 21.0 × 11.0 μ, but taking into consideration all the spore characters, the form seems quite distinct from other Melanconiums.

Pycnidial Stage

This was found only in cultures—in peptone agar in large numbers, and in oat agar (p. 421); in the latter case developing at about the same time as the conidia and surrounded by them. The pycnidia are black to the naked eye, spherical or irregular in shape, 150–400 μ in diameter, with

FIG. 2. Conidia: a, showing different forms, × 600: b, showing granular content or droplets, × 1000.

a rounded apical pore (sometimes 2 pores if of irregular shape), and partly sunk in the medium, being surrounded by a dense felt of pale smoke-gray mycelium. When placed in water, enormous numbers of tiny, oblong, hyaline pycnospores, (Fig. 3, a) about 1 × 3 μ, issue from the openings.

Ascogenous Stage

On Oct. 9, 1920, some of the dead twigs of a living butternut at Hamden, Conn., were observed to be not only plentifully covered with *Melanconium oblongum* pustules, but in and about these same pustules was what appeared to be the perfect stage in cracks in the bark (Pl. XX, fig. 10 and text fig. 4). The twigs were incubated in a moist chamber, and under these conditions, groups of long, black, hairlike structures, the necks of perithecia, grew out from the cracks (Pl. XX, fig. 11). Mature spores, shot out from the asci through these necklike openings,

were caught on slides suspended above, and sowings were made in oat agar on petri dishes under sterile conditions. These cultures matured typical conidia of *Melanconium oblongum* in 22 days. On December 2, the experiment was repeated, and on January 3, 1921, conidia again appeared.

The perithecia found at Hamden, Conn. were compared with the material at the New York Botanical Garden under the name of *Diaporthe Juglandis* E. & E. (p. 414) and found to be identical, with the exception, of course, of the long, extruded necks which had developed under cultural conditions.

On October 7, 1922, we received specimens labelled *Diaporthe Juglandis* E. & E. recently collected on butternut in Rhode Island by Prof. J. Franklin Collins. Examination showed that these also were the perfect stage. In October, 1922, the writer again collected the perfect stage at

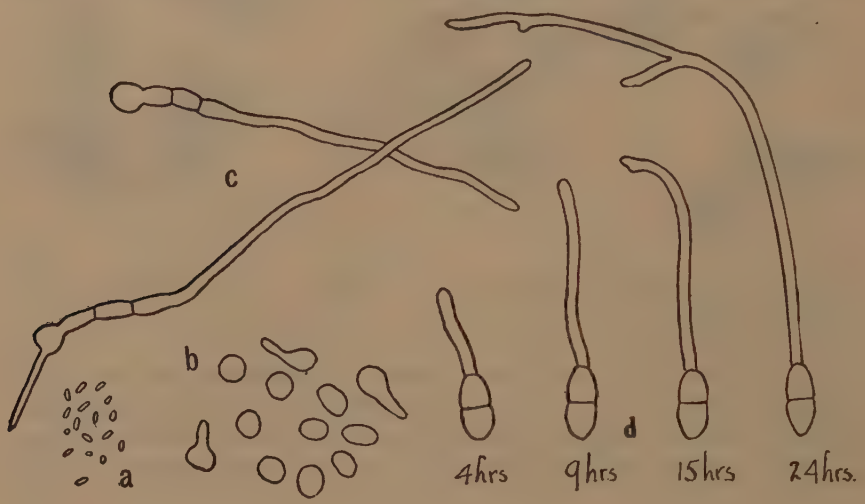

Fig. 3. Pycnospores; a, freshly ejected from pycnidia; b, 15 hrs. later, commencing germination; c, 18 hrs. after a, showing well developed germ tubes, × 500; d, germinating ascospore, × 500.

Hamden, Conn. It is extremely inconspicuous, being located in small cracks in the bark which when not as numerous as in the twig figured (Pl. XX, fig. 10), might pass for old lenticels.

Agar Cultures

Turnip juice agar, oat agar, potato juice agar, peptone agar, and butternut twig agar were all used as media, but for the production of

spores the oat agar gave by far the best results. This was somewhat modified from Dr. Clinton's formula (See Rept. Conn. Agr. Expt. Sta. **1909–10**: 760ff) as follows: fifty grams of rolled oats were cooked in 300 cc. of water in a double boiler for about one half hour, then pressed through cheesecloth or a fine wire strainer, 12 g. of agar added, and enough water to bring the whole volume up to 1000 cc. After boiling in the autoclave to melt the agar, titration showed the medium to be nearly neutral. After sterilization, the medium was opaque and nearly smoke gray (15, Pl. XLVI).[1]

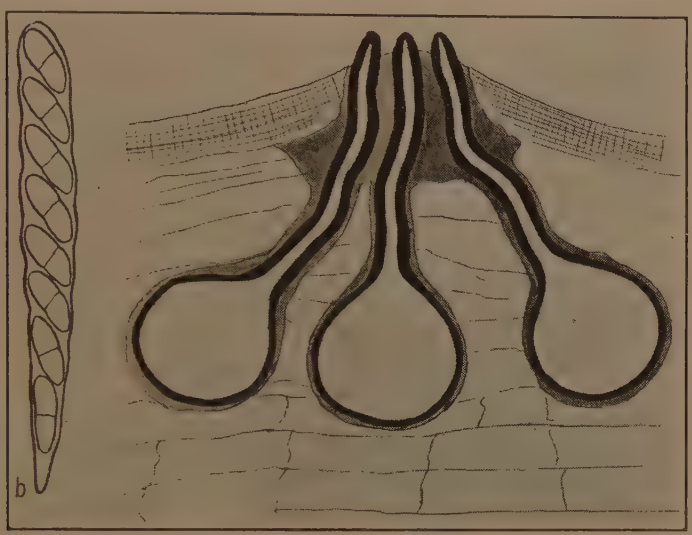

Fig. 4. a, Diagram of perithecia in bark, × 45; b, ascus with spores, × 550.

Cultures were derived from a single spore and obtained by the isolation and transplantation method, as follows. About two days after germination in the isolation petri dishes, the tiny cultures could readily be distinguished by holding the petri dish up to the light, one selected which seemed remote from any impurity, and then its origin from only one spore confirmed by examination of the petri dish directly on the stage of the microscope. Transplantation with a sterile scalpel to a dish of sterile medium was then a simple matter. Contamination in this process was inevitable, but if a considerable number of transplants were made, some would prove pure.

[1]Roman numerals refer to plates in Ridgway's Color Standards and Nomenclature (15).

The fungus shows marked differences of behavior in the different media, but only its characteristics in the oat agar will be described in detail. The young mycelium (3 days old) is an ochraceous-buff (15, Pl. XV), which deepens (8 days) into an ochraceous-orange (15, Pl. XV), and at length (2 weeks) assumes shades varying from olive-ocher to yellowish olive (15, Pl. XXX) mottled with mummy brown (15, Pl. XV). Tisdale (21) notes the presence of green shades in his cultures of Melanconium from tomatoes. Later, the color becomes more uniformly dark, and aerial mycelium, which up to now has been scanty, develops sparsely. In about 3 weeks a curious metallic luster appears on the surface of the medium, the center of the culture becoming nearly black. Small fluffy mounds of a smoke gray (15, Pl. XLVI) mycelium develop, in which the apparently coal black masses of conidia appear, like drops of thick ink. These changes vary somewhat, both qualitatively and quantitatively, and in time intervals, in different cultures, depending perhaps on the thickness of the medium, amount of moisture, temperature, etc.; but the sequence of colors is fairly uniform. In turnip juice agar and in potato agar, the mycelium has a tendency to wrinkle the surface of the medium. It should be noted that in all the cultures the growth was extremely slow, as compared with forms like Penicillium and Rhizopus.

Cultures on Sterile Butternut Twigs

These were cut into sections a few inches long, placed with a little distilled water in test tubes, sterilized, and inoculated with the fungus in the hope that the ascospore stage would develop on them. In some twigs after a little more than three weeks, conidial pustules appeared in the ordinary way, as small black pustules distributed in the bark. In other cases, the bark was fairly well covered with small cushions of mycelium (1–2½ mm. in diameter) of the characteristic olive shades—varying from a light olive gray (15, Pl. LI) to a deep grayish olive (15, Pl. XLVI) when old (Pl. XX, fig. 12). In about six weeks small, inky-black drops of conidia began to appear in these. This difference in behavior of these two sets of twigs was due perhaps to different moisture conditions. No perithecia developed in either case.

Germination of Spores

Conidia germinate readily in oat, potato, or peptone agar in 10–12 hours at laboratory temperature (Fig. 1, b). In tap water the germination is slight—one or two tubes in each drop—and in distilled water only in rare cases can germ tubes be seen.

Pycnospores sown in oat agar commence to swell and eventually assume enormous proportions, compared to their original size. Beginning as very tiny spores 1 × 3 μ, at the time when the germ tube appears they are about 6 μ in diameter, having taken on a more rounded form somewhat resembling yeast plants. Their volume thus increases nearly 40 fold (Fig. 3, a, b). A similar pregermination swelling occurs in the pycnospores of *Endothia parasitica* (1, p. 8), where it amounts to more than 85 times the volume of the resting spore. Germ tubes were put forth from the Melanconis pycnospores in from 15 to 16 hours at laboratory temperature. (Fig. 3, c.)

Ripe ascospores caught by the method described above (p. 421) and sown in oat agar germinated readily in 12–13 hours at laboratory temperature (Fig. 3, d). Germination occurred also in distilled water and in tap water, but it was poor—less than 1 per cent. Mycelium grown in oat agar from these ascospores produced typical conidia of "*Melanconium oblongum*" in from 3 to 4 weeks.

PATHOGENICITY OF THE FUNGUS

Data Based on Field Work

1. The fungus is constantly associated with this disease in the field. Nearly all wild butternuts of twenty years or more are affected to a greater or less extent. Trees of this age which do not show the conidial pustules, or the marks left by them on dead twigs (Pl. XX, fig. 7), are few indeed. The writer has yet to see a tree more than twenty years of age, whose limbs are accessible, on which in some part, the pustules of the fungus or traces of them, do not appear.

2. The proximal limit of the dead area on twigs or branches has been marked in the field and watched from year to year. This method has demonstrated the gradual encroachment of the fungus on healthy tissue, and subsequent appearance thereon of conidial pustules.

3. Records of 31 trees in a forest at West Hartford, Conn., have been kept since 1919, or through four growing seasons. These records show every year an increased number of branches infected with Melanconis. In some cases where the trees are vigorous, the number of healthy branches developing more than offsets those diseased; in others, the reverse is the case, while in others the total leaf area remains about the same from year to year.

4. The case in which the number of branches dying from this disease each year exceeds the number of new shoots developing, is illustrated by a particular tree in Connecticut which has been under observation for the last 16 years, and because it is considered a typical example of the progress of the disease, will be described here in some detail. Plate XIX, figures 1 and 2, which are photographs taken August 6, 1920 and July 30, 1922, show the course of the disease for a two year period. It is evident that the foliage in the later photograph is much more sparse, and a careful examination of the two figures shows that this is due to the death of many of the small leafy branches seen in the earlier photograph. For example, A, a branch of recent origin, formed in a sucker-like way at the base of a limb which has been gradually dying from the tip downward, is entirely dead in 1922. Similar sucker-like branches at B, bearing foliage in 1920, are now dead. At C, where a limb divides into three parts, a conspicuous growth has taken place in the two years, with development of many small branches, but few remain alive, so that the sum total of the foliage in 1922 is less than in 1920. On all dead branches which were accessible the Melanconis pustules were seen. The soil and light conditions for this tree are exceptionally good, so that there is no evident "physiological" reason for its condition. The fungus gained entrance probably through some dead or weakened branch perhaps 20–25 years ago, and the host has been undergoing a slow death ever since. Obviously only a short lease of life remains.

Inoculations of Healthy Twig Cuttings

As reported briefly in the preliminary note on this disease (8) vigorous cuttings of butternut were obtained in March, 1919, from wild trees, brought into the greenhouse and placed in jars of water. Fifty-nine inoculations with Melanconis conidia were made on 19 cuttings. Three more were used for checks, 8 incisions in all being made on these. On May 1 there were 33 infections, 19 of which showed the Melanconis conidial pustules, while 26 of the inoculations had healed and were apparently uninfected. Checks remained uninfected. A similar series on black walnut showed negative results on May 1.

In a second experiment of like nature, using twigs of butternut, Japanese walnut, and black walnut, the results were inconclusive because most of the cuttings soon withered and died. However, the black

walnut unfolded buds and continued healthy for some time, and this experiment is noteworthy mainly for the fact that out of 28 inoculation on this species, 5 developed the conidial pustules of Melanconis.

Inoculations of Forest Trees

1. In the spring of 1919 the branches and trunk of a young tree of butternut, about 5 inches in diameter, breast-high, were inoculated at 10 different points with a pure conidia-bearing culture of Melanconis. No careful record of the results was kept, but the inoculated branches were observed at irregular intervals and were eventually found in many cases to be plentifully besprinkled with the Melanconis conidia. The inoculations in the trunk healed over, and therefore in August, 1922, the tree was cut down to determine whether there was any internal result from these trunk inoculations. The incisions where the spores had been placed were found to be covered with four rings of healthy wood, but it was evident from the appearance of a dark discoloration that the fungus *had advanced both up and down in the trunk along the line of the 1919 ring of wood.* To determine the extent of this advance the trunk was cut into lengthwise and transverse sections which were left lying on the ground. On returning to the spot two months later, large numbers of the conidial pustules of Melanconis were found in the bark of these sections. This behavior of the fungus in the wood is most important to an understanding of its relations with its host, and will be discussed more in detail later.

2. A more careful series of inoculation experiments was commenced in July, 1919, at Hamden, Connecticut. Fifteen trees of *Juglans cinerea* of various ages were inoculated with conidia and the results noted at three inspections in the same year—August, September, and November. Inspections were made also in March, July, and November, 1920; in July 1921; and in July, 1922; so that these experiments cover a period of three years. One hundred inoculations and 20 controls were made.

In table 1 only the inoculations with positive results are given. With 8 exceptions, all others were made on large branches and on trunks ranging from 2 to 11 cm. in diameter, and externally, at least, gave no evidence of infection. During the first month no pustules had appeared, although according to field notes, in No. 62 there was an indication of slight elevations in the bark. By the second month, 3 of the inoculations gave positive results; in the fourth month, 5; at the expiration of one year, 17; two years, 24; and three years, 36. In the spring of 1922, however, a forest fire weakened trees II and III, with the result that the

TABLE 1—*Results of inoculations with Melanconis Juglandis on Juglans cinerea.*

No. of tree	No. of inoculation	Part of tree inoculated	Diameter in cm. at point of inoculation	Time of appearance of spore pustules (shown by + sign)				
				2 mos.	4 mos.	1 yr.	2 yrs.	3 yrs.
II	6	branch	1.4			+	+	+
"	7	"	1.5					+
"	8	"	1.2				+	+
"	9	"	1.3					+
III	10	trunk	2.1					+
"	11	"	2.0					+
"	12	"	2.0					+
"	13	branch	1.0				+	+
"	14	trunk	1.6					+
"	15	branch	0.7					+
"	16	"	0.9					+
VI	39	"	1.8			+	+	+
VII	40	trunk	12.5			+	+	+
"	41	"	12.5			+	+	+
"	42	"	12.0			+	+	+
"	43	"	11.5			+	+	+
"	44	"	11.5			+	+	+
IX	55	branch	0.3				+	+
"	56	"	0.9			+	+	+
"	57	"	0.4			+	+	+
"	58	"	0.4			+	+	+
"	59	"	0.9			+	+	+
"	60	"	0.5			+	+	+
X	61	"	0.8		+	+	+	+
"	62	"	0.4	+	+	+	+	+
"	63	"	0.4	+	+	+	+	+
"	64	"	1.0					+
"	65	"	0.8					+
"	66	"	0.4					+
"	69	"	0.9					+
"	70	"	0.4				+	+
"	71	"	0.5				+	+
"	72	"	1.0	+	+	+	+	+
"	73	"	0.6		+	+	+	+
XI	74	"	0.9				+	+
"	76	"	0.8				+	+
Total infections showing spore pustules				3	5	17	24	36

fungus appeared on nearly every branch, and taking this into consideration, it is fairly accurate to place the amount of apparent infection at about 33⅓ per cent.

Of the 20 controls, four were made on trees II and III on healthy branches which died in 1922 when these trees became entirely infected. This was also the case with one made on tree VII which died completely in 1920, as noted below. The other controls, except in cases where they were too near inoculations, remained uninfected.

Several peculiarities in the results of these inoculations are noteworthy. First of all, in most cases the living bark around the lesion healed promptly, giving the impression that the fungus was not a pathogen at all. By degrees, however, an inoculated twig would turn a reddish brown, and, if in the growing season, would drop its leaves gradually, the pustules finally appearing as small elevations and later breaking through the bark. In such cases, even the callus which had healed over the incisions became covered with acervuli (Pl. XX, fig. 6).

Secondly, in nearly every case except, for example, in tree VII, the spore pustules appeared on *small branches* not more than 2 cm. in diameter—in the majority of cases, 1 cm. or less.

Finally, if the host had already been weakened, the fungus grew rapidly, as in tree VII. Here the organism grew rapidly from the start in the thick, living bark of the trunk, behaving as does *Endothia parasitica* on chestnut, although no spore pustules could be distinguished for about a year. Tree VII was of fair dimensions, about 5 inches in diameter, breast-high, but growing in a dense forest where it had been suppressed and badly diseased at the top, so that it was in a weakened condition. By the end of four months one inoculation, for example, showed 30 cm. of fungous growth upward, 15 cm. downward, and 7 and 20 cm. from the sides of the point of inoculation. In the bark thus killed, spore pustules developed, (Pl. XX, fig. 4), the only case seen where pustules occurred in the thick rough bark of the trunk. The tree died entirely in 1920 directly from the girdling action of the fungus.

In the case of trees II and III, already mentioned as weakened by a forest fire in the spring of 1922, the conidia appeared on all the inoculated branches soon after the fire occurred. A discussion of these peculiarities will be postponed for the present (p. 431).

Inoculations of Seedlings

Although the inoculations on forest trees brought out several new features regarding the operations of the fungus, it was felt that as regards defining its responsibility in the causation of the disease they lacked

conclusiveness. Among other objections which might be brought forward was the fact that in all of the larger trees selected Melanconis already existed on one or more of the branches, although remote from the inoculations. For it had been found practically impossible to locate trees of any size in the forest which did not show the fungus in some portion.

In order to obtain a more clear cut demonstration it was decided therefore to inoculate seedlings that were entirely free from the fungus, and a new set of experiments was commenced on these in December, 1921, in the conservatories of the Brooklyn Botanic Garden. Through the kindness of Mr. Willard Bixby, the well-known Long Island nut-grower and horticulturist, 45 two or three year old Juglans seedlings were secured, distributed about equally among the four species, *J. regia*, *J. nigra*, *J. cinerea*, and *J. Sieboldiana*. These young trees were potted in one-half loam and one-half leaf mold and placed first in a cool greenhouse, and later in one of moderately warm temperature. Under the influence of the latter, by March 1, 1922 all were in full leaf and growing vigorously.

One thing brought out in clear relief by the previous experiments was the indication that *Melanconis Juglandis* is a weak parasite, attacking rapidly only when its host is weakened. It was therefore decided to weaken some of the seedlings of each species, previous to inoculation, in various ways. Thus, two or three of each species, with their roots carefully washed and uninjured, were transferred to pots of clean washed sand; these and all others, with the exception of a first group (Table 2, Group 1) which were treated to optimum conditions of soil, water and light, were placed beneath the greenhouse bench where the light was poor, and watered sparingly. In addition, those in sand were pruned repeatedly beginning March 1, and continuing up to the time of inoculation (Table 2, Group IV). Another group (Table 2, III) was similarly treated except that they remained in good soil. Group II was similar to III except that there was no pruning. No seedlings of *J. nigra* were placed in Group II. In this way four lots of seedlings within each species (with the exception just noted) were developed under the conditions as stated in the first column to the left in table 2.

With one tree for a control in each of the sixteen groups, inoculations with Melanconis conidia were made on May 17, 1922. By June 12 one tree of *J. Sieboldiana* in class II was dead and well spotted with Melanconis pustules (Fig. 5). Table 2 gives the results to Mar. 1, 1923. Plate XX, figs. 8 and 13, from a seedling of *J. Sieboldiana*, show the progress of the disease from one inoculation down the stem to the junction of the

TABLE 2—*Summarized results of inoculations (May 17, 1922) with Melanconis Juglandis on Juglans seedlings.*

Physical conditions	Species	No. of seedlings	No. of inoc.	No. of seedlings showing development of spore pustules				
				Aug. 5	Sept. 1	Oct. 1	Nov. 15	Mar. 1
I Optimum.	*J. regia*	2	3					
	J. nigra	2	3					
	J. cinerea	3	4					
	J. Sieboldiana	2	2					
II Good soil, poor light, little water.	*J. regia*	2	2					
	J. cinerea	3	3			1	1	1
	J. Sieboldiana	2	2	2	2	2	2	2
III Good soil, poor light, little water, pruned.	*J. regia*	2	2					
	J. nigra	3	4		1	1	1	1
	J. cinerea	1	1					1
	J. Sieboldiana	2	2	1	1	1	1	1
IV Sand, poor light, little water, pruned.	*J. regia*	2	2					
	J. nigra	3	3					
	J. cinerea	3	4					
	J. Sieboldiana	1	2			1	1	1
Total		33	39	3	4	6	6	7

branch. The inoculation on the latter has apparently had no effect, but it is only a question of time when the fungus will descend below the origin of this branch and thus cut it off. None of the controls developed the disease, though one tree of *J. Sieboldiana* in class IV, in which the conditions are severe, has died. Although the results are not striking, they point clearly to the conclusion that the fungus is a weak parasite and that its attacks are most marked in a weakened host.

Leaving out the inoculations on healthy seedlings it will be seen that the amount of infection on the weakened seedlings is somewhat over 25 per cent. Incidentally it may be noted that the experiment brings out a marked susceptibility in *J. Sieboldiana*. Just why those in the sand have resisted the fungus so well is not clear. However, the fungus works slowly, as shown in the previous experiment (p. 426).

DISCUSSION OF THE RELATIONS OF FUNGUS AND HOST

The fact that in most of the inoculations on forest trees the incision apparently healed over, led the writer to believe at first that the fungus was not a pathogen, or at least only a very weak one; for one had to admit that it "took" very readily in the living bark of tree VII (p. 427). The comparatively sudden death of whole twigs after two months, followed by an increasing number of dying inoculated twigs and small branches each year up to the termination of the experiments, led very early in the work to a field investigation of the inoculations. It was

Fig. 5. Showing results of inoculation of seedling of *Juglans Sieboldiana* grown in good soil, but weakened by poor light and little water. Check plant at right. Spore pustules show on stem of inoculated plant at left. × about $\frac{1}{5}$.

found that under the callus a small, black area extended radially into the wood and thence had proceeded up and down the twig or branch a distance depending on the length of incubation. In both inoculated branches and twigs diseased from natural infections this black column, overlaid by healthy wood and bark, has been followed well into the trunk. In a small twig, it is conceivable that this fungal invasion, necessarily hindering the upward flow of liquids in the twig, would eventually result in so weakening it that the fungus will readily advance outward to cambium and bark—hence the death of the twig and sub-

sequent appearance of spore pustules. It is of course possible that the killing is due to the presence of some toxic substance secreted by the fungus or formed by the interaction of host and parasite, as Clayton (3) believes is operative in the tomato wilt. In the case of the larger twigs, naturally a longer time would be required for the lethal action of the fungus, whatever its *modus operandi*. In the trunk, in the case of a vigorous tree, the formation of the annual rings of wood goes on so rapidly as to cover up for a time the action of the fungus. Yet it may eventually reach a spot where small branches are given off, and advancing into these, slowly kills them. If the tree is weakened by drought or other unfavorable conditions such as fire (p. 427) the fungus advances more rapidly.

Sections of discolored wood have been stained, and show the presence of the fungous hyphae. The isolation of the fungus in culture media from such discolored wood has been extremely difficult and successful only in a few cases. Where segments of twigs with the discolored wood have been incubated in a moist chamber, they have invariably developed spore pustules. The case has already been described (p. 425) of the tree trunk inoculated in 1919, and cut in 1922, which showed the discolored wood covered by four layers of healthy wood, and developed the spore pustules after it had lain two months on the ground.

From the evidence presented in the foregoing it seems clear that we are dealing here with a weak parasite working slowly, or almost dormant, in vigorous trees—more rapidly in weak ones. After the lapse of many years it may gain the upper hand (Pl. XIX, figs. 1, 2) and the death of the tree is the result.

RELATIVE SUSCEPTIBILITY OF SPECIES OF JUGLANS

It will be seen by reference to the list of exsiccati and hosts that *Melanconis Juglandis* is by no means restricted to the butternut. Very probably the fungus may be found on any species of Juglans. But as far as our field observations go, pathogenic effects are most pronounced in *J. cinerea* and *J. Sieboldiana*, which is to be expected on account of the close relationship of those two species. In the experiments with healthy seedlings (p. 427), *J. Sieboldiana* proved extremely susceptible.

Although we have seen some trees of *J. nigra*, in the field, apparently seriously affected and showing all the symptoms of the trouble, we do not feel warranted at present, in view of the slight experience we have had with this species, in making any definite statement as regards its degree of susceptibility. But on account of its great economic importance

a thorough investigation is much needed. Likewise, regarding the susceptibility of *J. regia*, we know little.

As regards the western species of Juglans, we find no reference to a trouble like this. Thornber (20) states that the Arizona walnut, *Juglans major* Hell. does not appear to be troubled with any serious fungous disease. The walnut blight, so destructive to the Persian walnut, (*J. regia*) and its hybrids in California, is an entirely different disease. (18)

REMEDIAL MEASURES AND CONTROL

In the forest, any remedial or control measures are under present conditions impracticable. In view of the nature of this disease, as brought out by these studies, it is clear that trees with a large percentage of branches killed by Melanconis are in all likelihood trunk-infected. Their growth increment will necessarily be slow, the amount depending on the extent of infection. Such trees may as well be removed whenever practicable, as in improvement thinnings.

But in the case of orchard trees, or in parks and gardens, with proper care and vigilance the disease may be successfully combatted. In view of the fact that weakened trees in particular are attacked by the fungus, efforts should be made to maintain them in as healthy a condition as possible, by such sanitary measures as fertilizing, mulching, watering in dry seasons, pruning to increase vigor; and by avoidance of conditions which lead to winter injury, etc. The trees should be constantly watched, and all dead twigs pruned off as soon as discovered. It is hardly necessary to add that any twigs or branches showing fruiting bodies of Melanconis should be pruned off immediately, the cut being made some distance below the limit of the wood discoloration in the interior. All pruning wounds should be covered with paint or tar. After the fungus has penetrated the trunk, no remedy is practicable.[1]

SUMMARY

The evidence indicates that the Melanconis disease extends over the entire natural range of the butternut, and is one of its chief troubles; while the cultivated Japanese walnut (*J. Sieboldiana*) is likewise particularly susceptible. The disease is also of significance in its possible relations to other species of Juglans.

[1] Dr. Robert T. Morris states in correspondence, that "if blighted butternut trees are cut near the ground, and the stump sprouts grafted later with Persian walnut, black walnut, or heart nut scions, we are likely to obtain good crops from this species which are highly resistant to attacks from the Melanconium."

In trees which are previously weakened, the fungus makes rapid progress; otherwise the disease advances slowly. The evidence at hand does not appear to warrant the statement that there is danger of disappearance of the butternut.

In its first stages, the trouble is characterized by the appearance of dead limbs besprinkled with small black conidial acervuli. Occasionally these develop spore horns, but they are usually rounded or wartlike, and in wet weather like drops of thick ink.

This conidial stage has passed under the name of *Melanconium oblongum* Berk. The ascospore stage, which may appear later near these same pustules, has long been known as *Diaporthe Juglandis* E. & E. By culture work this is now definitely proved to be the perfect stage of *Melanconium oblongum* Berk. *Melanconis Juglandis* (E. & E.) comb. nov. is therefore proposed as the new name.

A pycnospore stage has appeared in cultures on both oat agar and peptone agar.

Inoculation experiments, extending over a period of more than four years, have demonstrated conclusively that the fungus is a weak parasite. Entering usually through small twigs by way of a wound, or through twigs already killed from some other cause, the mycelium grows slowly down through the wood, producing a black or dark discoloration, to the main branch and finally to the trunk. Thence any branch may become infected.

Ordinarily the progress of the disease is so slow that the leaves fall one by one, not producing any striking wilting or blighting effects. In final stages trees have a marked stagheaded aspect.

Diseased branches should be pruned off promptly some distance below the apparent infection, and the wounds tarred over or painted. After the fungus has penetrated the trunk, combative measures are useless.

For forest trees, no remedy is practicable under present conditions. Diseased trees should be removed during improvement thinnings.

The writer wishes to acknowledge his indebtedness to the many who have contributed assistance and advice in this work—particularly to the following: Mr. A. B. Seymour, Harvard University, Dr. Robert T. Morris, New York City; Rev. Sidney Wallace, Waterbury, Conn.; Dr. G. P. Clinton, Mr. E. M. Stoddard, and Mr. Walter O. Filley, of the Connecticut Agricultural Experiment Station; Mrs. Flora W. Patterson, Bureau of Plant Industry, Washington, D. C.; to Professor A. W. Evans of Yale University, and to the staff of the New York Botanical Garden for placing at his disposal the facilities of their respective institutions;

and to Dr. George M. Reed of the Brooklyn Botanic Garden for helpful suggestions.

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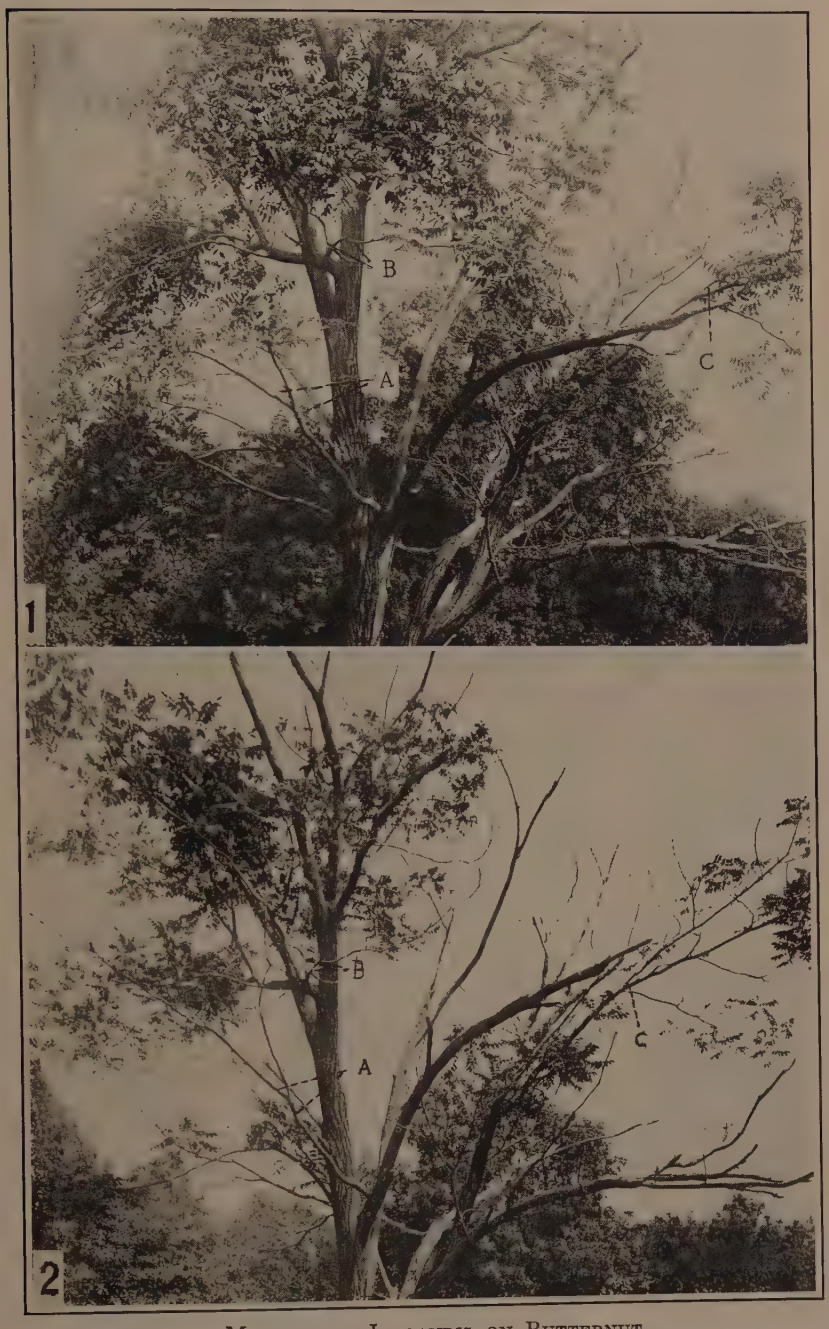
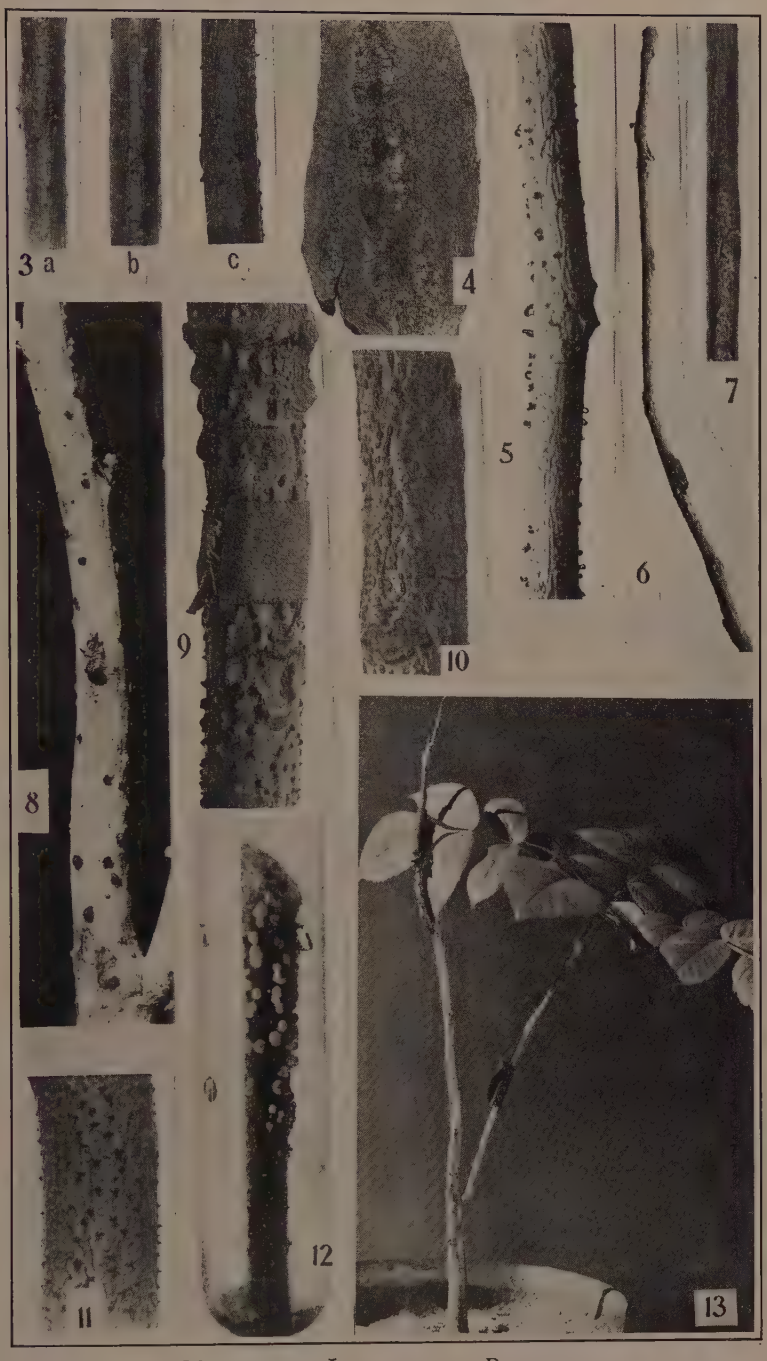


MELANCONIS JUGLANDIS ON BUTTERNUT



MELANCONIS JUGLANDIS ON BUTTERNUT

DESCRIPTION OF PLATES

PLATE XIX. MELANCONIS JUGLANDIS ON JUGLANS CINEREA

Figs. 1 and 2. A butternut tree at Hamden, Conn., showing the damage caused by the Melanconis disease in a two-year period. (1) Taken August 6, 1920; (2) Taken July 30, 1922. At A, B, and C are examples of branches which have died in the two-year interval. For detailed account see p. 424 of the text.

PLATE XX. MELANCONIS JUGLANDIS

Fig. 3. a, b, and c, successive stages in the development of the acervuli of the conidial stage of *Melanconis Juglandis* (E. & E.) comb. nov. on twigs of butternut: a, papillate swellings appearing; b, some of them burst open; c, warty, black masses of conidia. $\frac{4}{5}$ natural size.

Fig. 4. Acervuli, (the large black spots) on thick bark of trunk. $\frac{4}{5}$ natural size.

Fig. 5. Infected twig, incubated in a moist chamber, showing the extrusion of spores in horn-like masses from the acervuli. Such spore horns rarely occur in nature. $\frac{4}{5}$ natural size.

Fig. 6. Inoculated twig, showing where the inoculation was well healed over with a callus (about one inch from the base of photo); later, whole twig died as a result of growth of mycelium in interior, and numerous spore pustules appeared. This particular twig was a young green shoot at the time of inoculation. $\frac{2}{5}$ natural size.

Fig. 7. Part of old diseased twig showing holes in loose bark where spore pustules were located. $\frac{4}{5}$ natural size.

Fig. 8. Part of inoculated upright shoot of tree shown in Fig. 13, showing spore pustules in detail. Extent to which disease has apparently progressed is shown by irregular line below crotch. In the wood, however, the fungus has penetrated to the base of the tree, as was shown by subsequent examination. $\times$ 1.

Fig. 9. Branch of *Juglans Sieboldiana*, showing spore pustules appearing about the point of inoculation, some of them elongated or run together. About natural size.

Fig. 10. Ascogenous stage (not quite mature) appearing in cracks of butternut twig. $\times$ $1\frac{1}{5}$.

Fig. 11. Showing perithecial "necks" excessively developed, under moist chamber conditions. $\frac{4}{5}$ natural size.

Fig. 12. Pure culture on sterilized butternut twig, showing mycelial mounds where conidia will appear. About natural size.

Fig. 13. Seedling of *Juglans nigra* showing two inoculations, one of which has "taken," the fungus having progressed downwards. For further details see description of Fig. 8. About natural size.

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